βeta-lactam and Tetracycline Antibiotic Residues in Cow Milk in the Constantine region, Algeria

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Abstract

The aim of the present study was to investigate the βeta-lactam and tetracycline antibiotic residues in cow milk samples. A total of 122 samples of cow milk were collected from raw milk collectors (109 samples) and from a reconstituted pasteurized milk sales clerk (13 samples) in the Constantine region, Algeria and examined using the Beta Star Combo screening kit (Neogen, USA). Results indicates that 13 samples (10.66%) were positive for antibiotics residues: 12 (9.84%) for βeta-lactams (ten (8.20%) raw and two (1.64%) pasteurized milk samples) and only one (0.82%) for tetracyclines in a raw milk sample. It is evident that the Algerian consumer is not sheltered from the danger of antibiotic residues in milk and these inhibitor residues should constitute a constant concern for the dairy industry in Algeria. A control programme should be established.

Key words: βeta-*lactams; Tetracyclines; Residues; Cow milk; Constantine region*

Introduction

Antibiotics are a group of antimicrobials that are routinely administered to dairy animals as therapeutics and to prevent the outbreak of disease (Gaare et al., 2012). When dairy animals are treated with these groups of medications, residues may remain in the milk and may cause adverse effects on consumer health (Kabir and Niar, 2013) and milk processing. β-lactams and tetracyclines remain the most commonly used antibiotic families (Sachi et al., 2019).

In Algeria, the statistics on veterinary drug consumption, particularly antibiotics, are not precise. There is also a lack of information concerning milk contamination by βeta-lactams and tetracyclines antibiotic residues. Aggad et al. (2009) found the presence of growth bacteria inhibitors in 29% of analysed samples destined for human consumption in western Algeria. In central Algeria, Ben-Mahdi and Ouslimani (2009) found that 9.87% of

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samples were positive for antibiotic residues. Titouche et al. (2013) examined the incidence of antibiotic residues in raw milk in the Tizi-Ouzou area and found that 46.78% of 171 screened samples were positive. In Blida, Ouabdesselam et al. (2020) showed that 20.51% of 200 milk samples were positive for antibiotic residues. Their presence indicates a poor utilization especially the non-abidance of the withholding period (Abiola et al., 2005). The present study gives the first report on the presence of β-lactams and tetracycline antibiotic residues in raw and pasteurized cow milk in the Constantine region.

Materials and methods

From January to May 2019, a total of 122 cow milk samples were collected from raw milk collectors (109 samples) and from a reconstituted pasteurized milk sales clerk (13 samples) in the Constantine region of Algeria. All samples were examined for antibiotic residues using Beta-star Combo (Neogen Corporation, USA), which is a competitive receptor test in dipstick format that employs binding reagents linked to gold particles for the rapid detection assay at levels well below the maximum residue limit (MRL) of both βeta-lactam (amoxicillin, cloxacillin, oxacillin, ampicillin, penicillin G, dicloxacillin, nafcillin, cephapirin, cefalonium, cefoperazone, cefazolin, cefquinome, and ceftiofur) and tetracycline (chlortetracycline, oxytetracycline, tetracycline, and doxycycline) antibiotics, which are widely used in the treatment and prevention of disease in dairy cows, especially mastitis. This test is validated for use with raw and pooled cow milk samples. Using a syringe, 200 µL milk was extracted and added to the vial reagents. The vial was closed, returned and agitated to dissolve all solids. Once dissolved, the vial was incubated at 47.5°C±1 for 2 min in the block-heater. Following incubation, the dipstick was added and incubation continued for 3 min at the same temperature. In stage one, preliminary incubation of the binding reagents with milk containing antibiotics results in the interaction of the antibiotics with the binding reagents. In stage two, the solution is transferred into an immunochromatographic medium by which signal development occurs. The

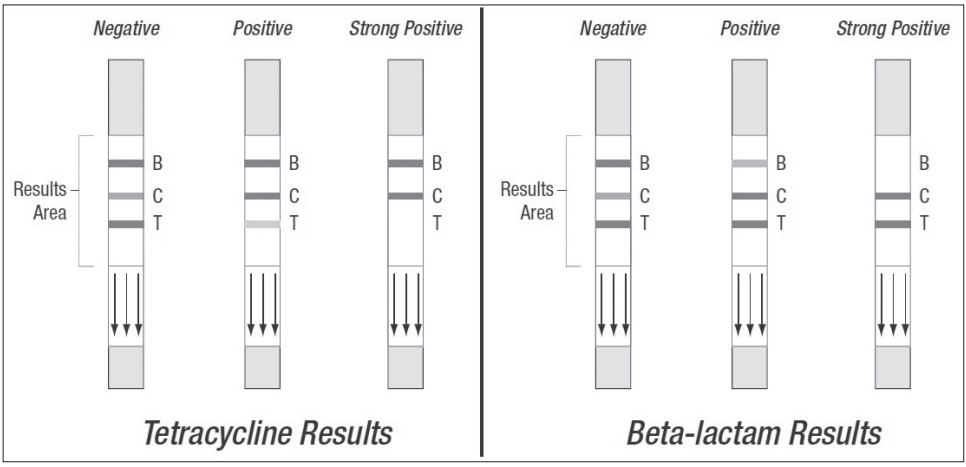

Figure 1. βeta-star Combo kit interpretation guide (Reybroecka and Ooghea, 2012)

Table 1. Results of the Beta Star Combo Test of Milk Samples

	Positive samples for tetracycline residues	Positive samples for βeta-lactam residues	Negatives samples	Total
Raw milk	1 (0.92%)	10 (9.17%)	98 (89.91%)	109
Pasteurized milk	0 (0%)	2 (15.38%)	11 (84.62%)	13
Total	1 (0.82%)	12 (9.84%)	109 (89.34%)	122

first line (lower) of this medium captures all the tetracyclines binding reagents that have not interacted with the tetracycline antibiotic during preliminary incubation. The second line (middle) on the stick serves as a control line to ensure proper function of the test itself, and also serves as a reference comparison for lines one and three. The third line (upper) of this medium captures all βeta-lactam binding reagents that have not interacted with any βeta-lactam antibiotic during preliminary incubation. At the end of incubation, we removed the stick from the vial and immediately compared the intensities of antibiotic test lines (lines 1 and 3) visually against the reference band. Negative: for each test line, the intensity of the test line is greater than or equal to the control line; no β-lactam or Tetracycline drug residue detected. Positive: if the intensity of the test line is less than the intensity of the control line, the milk sample is positive for the antibiotic (Reybroecka and Ooghea, 2012) (Figure 1).

Results

As shown in Table 1, of all the samples, 13 (10.66%) were positive for antibiotic residues (9.84% for βeta-lactam and 0.82% for tetracycline). Among the 109 raw milk samples, ten were positive for βeta-lactam residues and one for tetracycline. Among the 13 pasteurized milk samples, two were positive for βeta-lactam residues and no samples were positive for tetracycline residues.

Discussion

Milk containing antibiotics residues is unsuitable for human consumption (Hocine et al., 2021). The prevention of antibiotic residues is an important aspect of milk quality (Zeghilet et al., 2017), as milk must be exempt from antibiotics and/or other drug residues (Kouamé-Sina et al., 2010). All β-lactam and tetracycline antibiotic residues can be detected in milk using the Beta Star Combo kit. Among the 122 analysed samples, 10.66% contained antibiotic residues, and this is a high percent for cow milk in the Constantine region. This result indicates the excessive use of antibiotics in the treatment of dairy cows and it could be associated with the absence of elimination or isolation of milk producing by dairy cows treated with antibiotics. Thus, contaminated milk is mixed with uncontaminated milk and with the milk of other farms during collection. Antibiotic residues in reconstituted milk indicate that there is a lack of screening of these residues in imported milk powder (Zeghilet et al., 2017) and also confirm that heat treatments including pasteurization do not have any action against antibiotic residues (Fonseca et al., 2009; Schlemper and Sachet, 2017). The results clearly show that more samples contained β-lactams (9.84%) than tetracyclines (0.82), which was also reported in Iran (Noori et al., 2013), where positive results of 30% and 17.5% for β-lactam and tetracycline antibiotics residues were reported, respectively. Our results are also similar

to those observed in Palestine by Al Zuheir (2012), who reported the presence of β-lactam and tetracycline antibiotic residues in 22.2% and 18.7% from 18 and 14 raw milk samples, respectively. Contrary, in Romania, Pogurschi et al. (2015) reported that more samples were positive for tetracyclines residues (54 of 210 samples) than for β-lactam residues (12 of 210 samples). In central Algeria, 12.6% of samples were contaminated by inhibiting substances; β-lactams and Tetracyclines were present in 26.32% and 15.79% of the samples analysed, respectively (Baazize-Ammi et al., 2019).

In addition, in Algeria in the north central region, Mimoune et al. (2021a), found a contamination rate by antibiotic residues of 19.37%, 90% of positives samples were contaminated by beta-Lactamines and/or Tetracyclines residues.

β-lactams are the oldest but still one of the most widely used groups of antibiotics (Ghidini et al., 2002). β-lactam antibiotics are the most often used antibacterial substances in lactating cows for the treatment of bacterial infections (Dračková et al., 2009), especially mastitis which is one of the most common pathologies in dairy farming (Mimoune et al., 2021b), the important commercial and sanitary cost in milk production (Lamari et al., 2021), the most important cause of economic losses in the modern dairy industry (Benić et al., 2018), mastitis as the most expensive disease affecting dairy cattle worldwide and, therefore, the single largest cause of antibiotic usage in dairy herds in parenteral and intra-mammary therapy (Reybroecka et al., 2010). Consequently, it is the most common type of residues detected in milk (Gustavsson et al., 2004), responsible for approximately 95% of all milk antibiotic contamination (Movassagh and Karam, 2011a),

The presence of antibiotic residues in milk is not a problem unique to Algeria, and many countries suffer from this problem. In Iran, the prevalence of β-lactam residues in 150 individual cow raw samples and 150 pasteurized milk samples using the βeta-star test showed that 5.33% and 2.66% of samples were positive, respectively (Movassagh and Karam, 2011a,b). Also in Iran, Ghanavi et al. (2013) reported that 23.8 % of raw milk samples and 10.2% of pasteurized milk samples contained β-lactam antibiotic residues. Beldjil-Ali et al. (2013) examined the prevalence of antimicrobial residues in raw ewe milk in western Algeria, reporting that 4.76% of samples were positive. In Thailand, Wasiksiri et al. (2010) found that 22.72% of 44 raw goat milk samples were positive for antimicrobial substances. In Kosovo, Bytyqi et al. (2011) reported that 2.2% of 364 milk samples contained antibiotic residues. In India, Kumarswamy et al. (2018) used the Charm βeta-lactam tetracycline Combo Test and found that 2.42% and 1.82% of 165 raw cow milk samples were positive for β-lactam and tetracycline residues, respectively. In Nigeria, antibiotics residues were detected in 40.8% of fresh milk samples (Olatoye et al., 2016). In Italy, the IZSLER organization of Brescia tested 159543 milk samples using the microbial screening test and found that 549 (0.34%) were positive (Ghidini et al., 2002). In Benin, 83.9% and 16.5% of milk samples were positive for β-lactam and tetracycline antibiotis residues, respectively, using the twin sensor kit 020 (Mensah et al., 2019).

According to Nikolić et al. (2011), the most common causes of the persistence of antibiotics residues in milk is non-compliance of the withholding period for antibiotic excretion from the animal body, followed by overdose, use of banned antibiotics, and subsequent or deliberate addition of antibiotics to milk to prevent the multiplication of microorganisms that cause the deterioration of milk. Other causes may be the misuse of antibiotics for animal treatment in lactating cows, disease prevention, violation of

the withdrawal period, illegal use of antibiotics, use of antibiotics as growth promoters, etc. (Gaudin et al., 2004).

Antibiotic residues are often present in milk (Muji et al., 2018) and they are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers (Rama et al., 2016). Antibiotic residues in milk have strong public health significance as they cause adverse health effects in humans (Kumarswamy et al., 2018), such as allergic reactions, carcinogenicity, mutagenicity, teratogenicity, and drug resistance upon long-term exposure to antibiotics (Alimohammadi et al., 2020), which causes the failure of antibiotic therapy in clinical situations (Chowdhury et al., 2015) and contributes to a global health crisis (Rama et al., 2016). Antibiotic residues can also be responsible for hepatotoxicity, bone marrow toxicity, reproductive disorders (Brown et al., 2020), intestinal dysbiosis (Sykorova Goffova et al., 2012), immunopathological effects, autoimmunity and nephropathy (Nisha, 2008). For milk producers, the presence of antibiotic residues in milk causes major economic losses due to lost revenues (Kress et al., 2006). In addition to human health effects, the technological impact in the dairy sector must also be considered, since antimicrobial drugs can interfere with the production of dairy products, decreasing acid formation, reducing milk curdling and cause improper ripening of cheeses (Chiesa et al., 2020), resulting in financial losses in the dairy industry (Pogurschi et al., 2015). Antibiotic residues in animal products are also a factor of environmental and water pollution (Madougou et al., 2019). These reasons make milk containing antibiotic residues a harmful food whose sale is prohibited under Algerian law.

Conclusion

Antibiotic residues continue to be present in milk in Algeria, and the Algerian consumer is not sheltered from the danger of these residues. Antibiotic residues results clearly indicate that it is necessary to establish a regular system of control with the aim of protecting public health, to avoid problems in the dairy industry and finally to reduce waste milk caused by their presence.

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Ostatci β-laktamskih i tetraciklinskih antibiotika u kravljem mlijeku na području Konstantina, Alžir

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Cilj je ove studije bio ispitati ostatke β-laktamskih i tetraciklinskih antibiotika u uzorcima kravljeg mlijeka. U tu su svrhu prikupljena 122 uzorka kravljeg mlijeka od otkupljivača sirovog mlijeka (109 uzoraka) i od prodavača rekonstituiranog pasteriziranog mlijeka (13 uzoraka) na području Konstantina u Alžiru, a ispitani su pomoću Beta Star Combo kompleta za testiranje (Neogen, SAD). Rezultati su pokazali da je od svih analiziranih uzoraka trinaest (10,66 %) bilo pozitivno na ostatke antibiotika, dvanaest (9,84 %) na β-laktame (deset (8,20 %) sirovog mlijeka i dva (1,64 %) pasteriziranog mlijeka) i samo jedan uzorak (0,82 %) na tetracikline u sirovog mlijeka. Očito je da alžirski potrošači mlijeka nisu zaštićeni od opasnosti ostataka antibiotika u mlijeku pa bi ostatci tih inhibitora trebali biti razlog zabrinutosti za mliječnu industriju u Alžiru te je potrebno uspostaviti jači program kontrole.

Ključne riječi: β-laktami, *tetraciklini, ostatci, kravlje mlijeko, područje Konstantina*